

## **Mag-Bind® Bacterial DNA 96 Kit**

M2350-00	1 x 96 preps
M2350-01	4 x 96 preps
M2350-02	20 x 96 preps

**April 2012**



# Mag-Bind® Bacterial DNA 96 Kit

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Manual Revision: April 2012



# Introduction and Overview

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## Introduction

The Mag-Bind® Bacterial DNA 96 Kit allows rapid and reliable isolation of high-quality genomic DNA (gDNA) from a wide variety of bacterial species. Up to 0.5 mL gram-positive or gram-negative bacterial culture can be processed each time. The key to the system is Omega Bio-tek's proprietary Mag-Bind® Particles CND that reversibly bind DNA under optimal conditions allowing proteins and other contaminants to be removed. DNA is easily eluted with deionized water or low salt buffer.

NOTE: Mag-Bind® Bacterial DNA Kit will isolate all cellular DNA, including plasmid DNA.

## Overview

If using the Mag-Bind® Bacterial DNA 96 Kit for the first time, please read this booklet to become familiar with the procedures. After bacterial cells are collected from culture or picked from an agar plate, the bacterial cell wall is removed by two digestion steps: first with Lysozyme and second with Proteinase K. Following lysis, binding conditions are adjusted and the sample is mixed with Mag-Bind® Particles CND to bind the DNA. Two rapid wash steps remove trace salts and protein contaminants and DNA is eluted in water or low ionic strength buffer. Purified DNA can be used directly in downstream applications without the need for further purification.

### **New in this Edition:**

- Proteinase K is now supplied in a liquid form eliminating the step to resuspend prior to use. Proteinase K Solution can also be stored at room temperature for 12 months.
- Proteinase Storage Buffer is no longer included in the kit.

## Kit Contents

Product	M2350-00	M2350-01	M2350-02
Purifications	1 x 96 preps	4 x 96 preps	20 x 96 preps
Mag-Bind® Particles CND	1.1 mL	4.2 mL	21 mL
MB1 Buffer	25 mL	100 mL	500 mL
MB2 Buffer	3 mL	12 mL	60 mL
MSL Buffer	25 mL	100 mL	500 mL
SPM Wash Buffer	30 mL	150 mL	2 x 300 mL
Elution Buffer	25 mL	100 mL	500 mL
Lysozyme	120 mg	480 mg	2.4 g
Proteinase K Solution	2.5 mL	10 mL	50 mL
RNase A	550 µL	2.2 mL	5 x 2.2 mL
User Manual	✓	✓	✓

## Storage and Stability

All components of the Mag-Bind® Bacterial DNA 96 Kit, except the RNase A and lysozyme, can be stored at 22-25°C and are guaranteed for at least 12 months from the date of purchase. Store the Mag-Bind® Particles CND and RNase A at 2-8°C. Proteinase K Solution can be stored at room temperature for 12 months. For long term store (>12 months) store at 2-8°C. Once reconstituted, Lysozyme must be stored at -20°C. Under cool ambient conditions, a precipitate may form in the MSL Buffer or MB2 Buffer. In case of such an event, heat the bottle at 37°C to dissolve.

## Preparing Reagents

Prepare a stock solution of Lysozyme (50 mg/mL) as follows and aliquot. Store each aliquot at -20°C and thaw before use.

Kit	Elution Buffer to be Added
M2350-00	2.4 mL
M2350-01	9.6 mL
M2350-02	48 mL

Dilute SPM Wash Buffer with ethanol (96-100%) as follows:

Kit	Ethanol (96-100%) to be Added
M2350-00	70 mL
M2350-01	350 mL
M2350-02	700 mL per bottle

# Mag-Bind® Bacterial DNA 96 Kit Protocol

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## Mag-Bind® Bacterial DNA 96 Kit Protocol - DNA from Culture or Agar Plates

### Materials and Reagents to be Supplied by User:

- Centrifuge with rotor and adaptor for microplates
- Magnetic Separation Device (Cat# MSD-01)
- 1.2 or 2 mL deep-well plates
- Vortexer and sealing films
- Ethanol (96-100%)
- Multi-channel pipettor and tips
- Shaking water bath, incubator, or heat block capable of 37°C and 60°C
- Optional: Lysostaphin (1 mg/mL)

### Before Starting:

- Prepare SPM Wash Buffer according to the Preparing Reagents Section on Page 4
- Prepare Lysozyme stock solutions according to the Preparing Reagents Section on Page 4
- Set incubator or heat block to 37°C
- Set incubator or heat block to 60°C

### 1. Preparing Cells

- Cells from Culture:
  1. Transfer 0.5 mL bacterial culture into each well of a 1.2 or 2 mL deep-well plate.
  2. Centrifuge at 4,000 x *g* at room temperature for 10 minutes.
  3. Carefully aspirate and discard the media without disturbing the cell pellet.
  4. Proceed to Step 2.
- Cells from an Agar Plate
  1. Add 90 µL MB1 Buffer and 10 µL Lysozyme into each well of the sample plate.
  2. Add one colony into each well and mix thoroughly.
  3. Proceed to Step 3.

# Mag-Bind® Bacterial DNA 96 Kit Protocol

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**Note:** If you choose to vortex the samples in during the protocol below, make sure to seal the plate completely to avoid any loss of sample or cross-contamination.

2. Add 90  $\mu\text{L}$  MB1 Buffer and 10  $\mu\text{L}$  Lysozyme to each sample. Mix the sample thoroughly by pipetting up and down 20 times. Make sure the cells are fully resuspended.

**Note:** For some species of staphylococci, add 1-2  $\mu\text{L}$  lysostaphin (1 mg/mL). Lysostaphin is not supplied.

3. Incubate at 37°C for 10 minutes. Mix the plate 1-2 times during incubation by vortexing or pipetting up and down 20 times.

**Note:** The amount of enzyme required and/or the incubation time may need to be modified depending on the bacterial strain used. Complete digestion of the cell wall is essential for efficient lysis. Longer incubation time might yield better results.

4. Add 12  $\mu\text{L}$  MB2 Buffer and 20  $\mu\text{L}$  Proteinase K Solution. Mix thoroughly by vortexing or pipetting up and down 20 times.

5. Incubate at 60°C in a shaking water bath for 20 minutes for gram-negative bacteria, or 40-60 minutes for gram-positive bacteria.

**Note:** Usually no more than 1 hour is needed for bacterial lysis. If a shaking water bath is not available, incubate and shake plate every 20-30 minutes.

6. Add 5  $\mu\text{L}$  RNase A to each sample. Mix thoroughly by vortexing or pipetting up and down 20 times.

7. Incubate at room temperature for 5 minutes.

8. Add 135  $\mu\text{L}$  MSL Buffer and 10  $\mu\text{L}$  Mag-Bind® Particles CND. Mix thoroughly by vortexing or pipetting up and down 20 times.

9. Add 182  $\mu\text{L}$  ethanol (96-100%). Mix thoroughly by vortexing or pipetting up and down 20 times.



# Mag-Bind® Bacterial DNA 96 Kit Protocol

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10. Incubate at room temperature for 5 minutes.
11. Place the plate on a magnetic separation device to magnetize the Mag-Bind® Particles CND. Let sit for 10-15 minutes.
12. Aspirate and discard the supernatant. Do not disturb the Mag-Bind® Particles CND.
13. Remove the plate from the magnetic separation device.
14. Add 400 µL SPM Wash Buffer to each sample.  
**Note:** SPM Wash Buffer must be diluted with ethanol prior to use. Please see Page 4 for instructions.
15. Resuspend the Mag-Bind® Particles CND by vortexing or pipetting up and down 20 times. Incubate for 3 minutes at room temperature. Mix by vortexing or pipetting a few times during incubation.  
**Note:** Complete resuspension is required for adequate washing of the Mag-Bind® Particles.
16. Place the plate on the magnetic separation device to magnetize the Mag-Bind® Particles CND. Let sit at room temperature until the Mag-Bind® Particles CND are completely cleared from solution.
17. Aspirate and discard the supernatant. Do not disturb the Mag-Bind® Particles CND.
18. Repeat Steps 13-17 for a second SPM wash step.
19. Leave the plate on the magnetic separation device for 5-10 minutes to air dry the Mag-Bind® Particles CND. Remove any residual liquid with a pipettor.
20. Remove the plate from the magnetic separation device.

# Mag-Bind® Bacterial DNA 96 Kit Protocol

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21. Add 200 µL Elution Buffer to elute DNA from the Mag-Bind® Particles CND. Resuspend the Mag-Bind® Particles by pipetting up and down 50 times or vortexing for 3 minutes.
  
22. Incubate 5-10 minutes at room temperature.  
  
**Note:** Incubation at 60°C rather than at room temperature will give a modest increase in DNA yield per elution.
  
23. Place the plate on the magnetic separation device to magnetize the Mag-Bind® Particles CND. Let sit at room temperature until the Mag-Bind® Particles CND are completely cleared from solution.
  
24. Transfer the cleared supernatant containing purified DNA to a clean plate. Store the DNA at -20°C.

# Mag-Bind® Bacterial DNA 96 Kit Protocol

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## Mag-Bind® Bacterial DNA 96 Kit Protocol - DNA from Viscous or Mucous Samples

### Materials and Reagents to be Supplied by User:

- Magnetic Separation Device (Cat# MSD-01)
- 1.2 or 2 mL deep-well plates
- Vortexer and sealing films
- Ethanol (96-100%)
- Multi-channel pipettor and tips
- Shaking water bath, incubator, or heat block capable of 37°C and 60°C
- DTT

### Before Starting:

- Prepare SPM Wash Buffer according to the Preparing Reagents Section on Page 4
- Prepare Lysozyme stock solutions according to the Preparing Reagents Section on Page 4
- Prepare fresh 0.15% DTT (w/v) solution in MB1 just prior to DNA extraction
- Set incubator or heat block to 37°C
- Set incubator or heat block to 60°C

**Note:** If you choose to vortex the samples in during the protocol below, make sure to seal the plate completely to avoid any loss of sample or cross-contamination.

1. Add 200  $\mu$ L of sample into a 96-well deep-well plate (1.2 or 2 mL).
2. Add 200  $\mu$ L MB1 Buffer with freshly prepared DTT solution. Incubate at 37°C until the sample can be pipetted.
3. Transfer 200  $\mu$ L sample into a new deep-well plate.
4. Add 20  $\mu$ L Lysozyme to each sample.
5. Incubate at 37°C for 10 minutes.

# Mag-Bind® Bacterial DNA 96 Kit Protocol

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6. Add 25  $\mu$ L MB2 Buffer and 20  $\mu$ L Proteinase K Solution. Mix thoroughly by vortexing or pipetting up and down 20 times.
7. Incubate at 60°C in a shaking water bath for 20 minutes for gram-negative bacteria, or 40-60 minutes for gram-positive bacteria.

**Note:** Usually no more than 1 hour is needed for bacterial lysis. If a shaking water bath is not available, incubate and shake plate every 20-30 minutes.

8. Add 5  $\mu$ L RNase A to each sample. Mix thoroughly by vortexing or pipetting up and down 20 times.
9. Incubate at room temperature for 5 minutes.
10. Add 245  $\mu$ L MSL Buffer and 10  $\mu$ L Mag-Bind® Particles CND. Mix thoroughly by vortexing or pipetting up and down 20 times.
11. Add 330  $\mu$ L ethanol (96-100%). Mix thoroughly by vortexing or pipetting up and down 20 times.
12. Transfer half of the sample volume into a round-bottom 96-well plate.
13. Place the plate on a magnetic separation device to magnetize the Mag-Bind® Particles CND. Let sit for 10-15 minutes.
14. Aspirate and discard the supernatant. Do not disturb the Mag-Bind® Particles CND.
15. Repeat Steps 12-14 until all of the Mag-Bind® Particles CND from the samples are collected.
16. Remove the plate from the magnetic separation device.
17. Add 400  $\mu$ L SPM Wash Buffer to each sample.

**Note:** SPM Wash Buffer must be diluted with ethanol prior to use. Please see Page 4 for instructions.

# Mag-Bind® Bacterial DNA 96 Kit Protocol

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18. Resuspend the Mag-Bind® Particles CND by vortexing or pipetting up and down 20 times.

**Note:** Complete resuspension is required for adequate washing of the Mag-Bind® Particles.

19. Place the plate on the magnetic separation device to magnetize the Mag-Bind® Particles CND. Let sit at room temperature until the Mag-Bind® Particles CND are completely cleared from solution.
20. Aspirate and discard the supernatant. Do not disturb the Mag-Bind® Particles CND.
21. Repeat Steps 16-20 for a second SPM wash step.
22. Leave the plate on the magnetic separation device for 5-10 minutes to air dry the Mag-Bind® Particles CND. Remove any residual liquid with a pipettor.
23. Remove the plate from the magnetic separation device.
24. Add 200 µL Elution Buffer to elute DNA from the Mag-Bind® Particles CND. Resuspend the Mag-Bind® Particles CND by pipetting up and down 50 times or vortexing for 3 minutes.
25. Incubate 5-10 minutes at room temperature.

**Note:** Incubation at 60°C rather than at room temperature will give a modest increase in DNA yield per elution.
26. Place the plate on the magnetic separation device to magnetize the Mag-Bind® Particles CND. Let sit at room temperature until the Mag-Bind® Particles CND are completely cleared from solution.
27. Transfer the cleared supernatant containing purified DNA to a clean plate. Store the DNA at -20°C.

# Mag-Bind® Bacterial DNA 96 Kit Protocol

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## Mag-Bind® Bacterial DNA 96 Kit Protocol - DNA from Urine

### Materials and Reagents to be Supplied by User:

- Centrifuge with rotor and adaptor for microplates
- Magnetic Separation Device (Cat# MSD-01)
- 1.2 or 2 mL deep-well plates
- Vortexer and sealing films
- Ethanol (96-100%)
- Multi-channel pipettor and tips
- Shaking water bath, incubator, or heat block capable of 37°C and 60°C
- Optional: Lysostaphin (1 mg/mL)

### Before Starting:

- Prepare SPM Wash Buffer according to the Preparing Reagents Section on Page 4
- Prepare Lysozyme stock solutions according to the Preparing Reagents Section on Page 4
- Set incubator or heat block to 37°C
- Set incubator or heat block to 60°C

**Note:** If you choose to vortex the samples in during the protocol below, make sure to seal the plate completely to avoid any loss of sample or cross-contamination.

1. Add 1 mL urine into each well of a 1.2 or 2 mL deep-well plate.
2. Centrifuge at 3,000 x *g* for 5 minutes.
3. Carefully aspirate and discard the supernatant.
4. Add 200 µL MB1 Buffer. Resuspend the pellet by vortexing for 20 seconds.
5. Add 20 µL Lysozyme to each sample.

**Note:** For some species of staphylococci, add 1-2 µL Lysostaphin (1 mg/mL). Lysostaphin is not supplied.

6. Incubate at 37°C for 10 minutes.

# Mag-Bind® Bacterial DNA 96 Kit Protocol

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7. Add 25  $\mu$ L MB2 Buffer and 20  $\mu$ L Proteinase K Solution. Mix thoroughly by vortexing or pipetting up and down 20 times.
8. Incubate at 60°C in a shaking water bath for 20 minutes for gram-negative bacteria, or 40-60 minutes for gram-positive bacteria.  
  
**Note:** Usually no more than 1 hour is needed for bacterial lysis. If a shaking water bath is not available, incubate and shake plate every 20-30 minutes.
9. Add 5  $\mu$ L RNase A to each sample. Mix thoroughly by vortexing or pipetting up and down 20 times.
10. Incubate at room temperature for 5 minutes.
11. Add 245  $\mu$ L MSL Buffer and 10  $\mu$ L Mag-Bind® Particles CND. Mix thoroughly by vortexing or pipetting up and down 20 times.
12. Add 330  $\mu$ L ethanol (96-100%). Mix thoroughly by vortexing or pipetting up and down 20 times.
13. Transfer half of the sample volume into a round-bottom 96-well plate.
14. Place the plate on a magnetic separation device to magnetize the Mag-Bind® Particles CND. Let sit for 10-15 minutes.
15. Aspirate and discard the supernatant. Do not disturb the Mag-Bind® Particles CND.
16. Repeat Steps 13-15 until all of the Mag-Bind® Particles CND from the samples are collected.
17. Remove the plate from the magnetic separation device.
18. Add 400  $\mu$ L SPM Wash Buffer to each sample.

**Note:** SPM Wash Buffer must be diluted with ethanol prior to use. Please see Page 4 for instructions.

# Mag-Bind® Bacterial DNA 96 Kit Protocol

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19. Resuspend the Mag-Bind® Particles CND by vortexing or pipetting up and down 20 times.

**Note:** Complete resuspension is required for adequate washing of the Mag-Bind® Particles.

20. Place the plate on the magnetic separation device to magnetize the Mag-Bind® Particles CND. Let sit at room temperature until the Mag-Bind® Particles CND are completely cleared from solution.
21. Aspirate and discard the supernatant. Do not disturb the Mag-Bind® Particles CND.
22. Repeat Steps 17-21 for a second SPM wash step.
23. Leave the plate on the magnetic separation device for 5-10 minutes to air dry the Mag-Bind® Particles CND. Remove any residual liquid with a pipettor.
24. Remove the plate from the magnetic separation device.
25. Add 200 µL Elution Buffer to elute DNA from the Mag-Bind® Particles CND. Resuspend the Mag-Bind® Particles by pipetting up and down 50 times or vortexing for 3 minutes.
26. Incubate 5-10 minutes at room temperature.

**Note:** Incubation at 60°C rather than at room temperature will give a modest increase in DNA yield per elution.
27. Place the plate on the magnetic separation device to magnetize the Mag-Bind® Particles CND. Let sit at room temperature until the Mag-Bind® Particles CND are completely cleared from solution.
28. Transfer the cleared supernatant containing purified DNA to a clean plate. Store the DNA at -20°C.



# Mag-Bind® Bacterial DNA 96 Kit Protocol

## Mag-Bind® Bacterial DNA 96 Kit Protocol - DNA from Body Fluids

### Materials and Reagents to be Supplied by User:

- Centrifuge with rotor and adaptor for microplates
- Magnetic Separation Device (Cat# MSD-01)
- 1.2 or 2 mL deep-well plates
- Vortexer and sealing films
- Ethanol (96-100%)
- Multi-channel pipettor and tips
- Shaking water bath, incubator, or heat block capable of 37°C and 60°C
- Optional: Lysostaphin (1 mg/mL)

### Before Starting:

- Prepare SPM Wash Buffer according to the Preparing Reagents Section on Page 4
- Prepare Lysozyme stock solutions according to the Preparing Reagents Section on Page 4
- Set incubator or heat block to 37°C
- Set incubator or heat block to 60°C

**Note:** If you choose to vortex the samples in during the protocol below, make sure to seal the plate completely to avoid any loss of sample or cross-contamination.

1. Add 100 µL sample into each well of a 1.2 or 2 mL deep-well plate.
2. Add 100 µL MB1 Buffer. Mix thoroughly by vortexing or pipetting up and down 20 times.
3. Add 20 µL Lysozyme to each sample and incubate at 37°C for 10 minutes.

**Note:** For some species of staphylococci, add 1-2 µL Lysostaphin (1 mg/mL). Lysostaphin is not supplied.

4. Add 25 µL MB2 Buffer and 20 µL Proteinase K Solution to each sample. Mix thoroughly by vortexing or pipetting up and down 20 times.

# Mag-Bind® Bacterial DNA 96 Kit Protocol

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5. Incubate at 60°C in a shaking water bath for 20 minutes for gram-negative bacteria, or 40-60 minutes for gram-positive bacteria.

**Note:** Usually no more than 1 hour is needed for bacterial lysis. If a shaking water bath is not available, incubate and shake plate every 20-30 minutes.

6. Add 5 µL RNase A to each sample. Mix thoroughly by vortexing or pipetting up and down 20 times.
7. Incubate at room temperature for 5 minutes.
8. Add 245 µL MSL Buffer and 10 µL Mag-Bind® Particles CND. Mix thoroughly by vortexing or pipetting up and down 20 times.
9. Add 330 µL ethanol (96-100%). Mix thoroughly by vortexing or pipetting up and down 20 times.
10. Transfer half of the sample volume into a round-bottom 96-well plate.
11. Place the plate on a magnetic separation device to magnetize the Mag-Bind® Particles CND. Let sit for 10-15 minutes.
12. Aspirate and discard the supernatant. Do not disturb the Mag-Bind® Particles CND.
13. Repeat Steps 10-12 until all of the Mag-Bind® Particles CND from the samples are collected.
14. Remove the plate from the magnetic separation device.
15. Add 400 µL SPM Wash Buffer to each sample.

**Note:** SPM Wash Buffer must be diluted with ethanol prior to use. Please see Page 4 for instructions.

# Mag-Bind® Bacterial DNA 96 Kit Protocol

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16. Resuspend the Mag-Bind® Particles CND by vortexing or pipetting up and down 20 times.

**Note:** Complete resuspension is required for adequate washing of the Mag-Bind® Particles.

17. Place the plate on the magnetic separation device to magnetize the Mag-Bind® Particles CND. Let sit at room temperature until the Mag-Bind® Particles CND are completely cleared from solution.
18. Aspirate and discard the supernatant. Do not disturb the Mag-Bind® Particles CND.
19. Repeat Steps 14-18 for a second SPM wash step.
20. Leave the plate on the magnetic separation device for 5-10 minutes to air dry the Mag-Bind® Particles CND. Remove any residual liquid with a pipettor.
21. Remove the plate from the magnetic separation device.
22. Add 200 µL Elution Buffer to elute DNA from the Mag-Bind® Particles CND. Resuspend the Mag-Bind® Particles by pipetting up and down 50 times or vortexing for 3 minutes.
23. Incubate 5-10 minutes at room temperature.  
**Note:** Incubation at 60°C rather than at room temperature will give a modest increase in DNA yield per elution.
24. Place the plate on the magnetic separation device to magnetize the Mag-Bind® Particles CND. Let sit at room temperature until the Mag-Bind® Particles CND are completely cleared from solution.
25. Transfer the cleared supernatant containing purified DNA to a clean plate. Store the DNA at -20°C.

# Mag-Bind® Bacterial DNA 96 Kit Protocol

## Mag-Bind® Bacterial DNA 96 Kit Protocol - DNA from Secretion Swabs (buccal or nasal swab)

### Materials and Reagents to be Supplied by User:

- Centrifuge with rotor and adaptor for microplates
- Magnetic Separation Device (Cat# MSD-01)
- 1.2 or 2 mL deep-well plates
- Vortexer and sealing films
- Ethanol (96-100%)
- Multi-channel pipettor and tips
- Shaking water bath, incubator, or heat block capable of 37°C and 60°C
- Optional: Lysostaphin (1 mg/mL)

### Before Starting:

- Prepare SPM Wash Buffer according to the Preparing Reagents Section on Page 4
- Prepare Lysozyme stock solutions according to the Preparing Reagents Section on Page 4
- Set incubator or heat block to 37°C
- Set incubator or heat block to 60°C

**Note:** If you choose to vortex the samples in during the protocol below, make sure to seal the plate completely to avoid any loss of sample or cross-contamination.

1. Add 280 µL MB1 Buffer in each well of a 1.2 or 2 mL deep-well plate.
2. Submerge a swab tip into each well.
3. Add 20 µL Lysozyme to each sample and incubate at 37°C for 10 minutes.

**Note:** For some species of staphylococci, add 1-2 µL Lysostaphin (1 mg/mL). Lysostaphin is not supplied.

4. Transfer 200 µL sample into a new deep-well plate.
5. Add 25 µL MB2 Buffer and 20 µL Proteinase K Solution to each sample. Mix thoroughly by vortexing or pipetting up and down 20 times.

# Mag-Bind® Bacterial DNA 96 Kit Protocol

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6. Incubate at 60°C in a shaking water bath for 20 minutes for gram-negative bacteria, or 40-60 minutes for gram-positive bacteria.

**Note:** Usually no more than 1 hour is needed for bacterial lysis. If a shaking water bath is not available, incubate and shake plate every 20-30 minutes.

7. Add 5 µL RNase A to each sample. Mix thoroughly by vortexing or pipetting up and down 20 times.
8. Incubate at room temperature for 5 minutes.
9. Add 245 µL MSL Buffer and 10 µL Mag-Bind® Particles CND. Mix thoroughly by vortexing or pipetting up and down 20 times.
10. Add 330 µL ethanol (96-100%). Mix thoroughly by vortexing or pipetting up and down 20 times.
11. Transfer half of the sample volume into a round-bottom 96-well plate.
12. Place the plate on a magnetic separation device to magnetize the Mag-Bind® Particles CND. Let sit for 10-15 minutes.
13. Aspirate and discard the supernatant. Do not disturb the Mag-Bind® Particles CND.
14. Repeat Steps 11-13 until all of the Mag-Bind® Particles CND from the samples are collected.
15. Remove the plate from the magnetic separation device.
16. Add 400 µL SPM Wash Buffer to each sample.

**Note:** SPM Wash Buffer must be diluted with ethanol prior to use. Please see Page 4 for instructions.

# Mag-Bind® Bacterial DNA 96 Kit Protocol

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17. Resuspend the Mag-Bind® Particles CND by vortexing or pipetting up and down 20 times.

**Note:** Complete resuspension is required for adequate washing of the Mag-Bind® Particles.

18. Place the plate on the magnetic separation device to magnetize the Mag-Bind® Particles CND. Let sit at room temperature until the Mag-Bind® Particles CND are completely cleared from solution.

19. Aspirate and discard the supernatant. Do not disturb the Mag-Bind® Particles CND.

20. Repeat Steps 15-19 for a second SPM wash step.

21. Leave the plate on the magnetic separation device for 5-10 minutes to air dry the Mag-Bind® Particles CND. Remove any residual liquid with a pipettor.

22. Remove the plate from the magnetic separation device.

23. Add 200 µL Elution Buffer to elute DNA from the Mag-Bind® Particles CND. Resuspend the Mag-Bind® Particles by pipetting up and down 50 times or vortexing for 3 minutes.

24. Incubate 5-10 minutes at room temperature.

**Note:** Incubation at 60°C rather than at room temperature will give a modest increase in DNA yield per elution.

25. Place the plate on the magnetic separation device to magnetize the Mag-Bind® Particles CND. Let sit at room temperature until the Mag-Bind® Particles CND are completely cleared from solution.

26. Transfer the cleared supernatant containing purified DNA to a clean plate. Store the DNA at -20°C.

## Troubleshooting Guide

Please use this guide to troubleshoot any problems that may arise. For further assistance, please contact the technical support staff, toll free, at **1-800-832-8896**.

Problem	Cause	Solution
Low DNA yields	Incomplete resuspension of Mag-Bind® Particles CND	Resuspend the Mag-Bind® Particles CND by vortexing before use
	Inefficient cell lysis	<ul style="list-style-type: none"> <li>• Increase the Lysozyme incubation time</li> <li>• Increase the Proteinase K digestion time</li> </ul>
	SPM Wash Buffer is not prepared correctly	Prepare the SPM Wash Buffer by adding ethanol according to the instructions
	Loss of Mag-Bind® Particles CND during operation	Do not aspirate the Mag-Bind® Particles CND during pipetting
No DNA eluted	SPM Wash Buffer not diluted with ethanol	Prepare the SPM Wash Buffer by adding ethanol according to the instructions
Problem with downstream applications	Ethanol carry over	Make sure to remove all SPM Wash Buffer during the Mag-Bind® Particles CND drying step

## Ordering Information

The following components are available for purchase separately.

Call Toll Free at 1-800-832-8896

Buffer (Size)	Part Number
Elution Buffer, 100 mL	PDR048
Elution Buffer, 500 mL	PD089
MSL Buffer, 100 mL	PD070
SPM Wash Buffer, 40 mL	PS014
RNase A, 400 $\mu$ L	AC117
RNase A, 5 mL	AC118
Magnetic Separation Device for Microplates	MSD-01
1.2 mL Deep-well Plates	SSI1780
Sealing Film	AC1200
Proteinase K Solution	AC116

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PCR is a patented process of Hoffman-La Roche. Use of the PCR process requires a license.



**Notes:**

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**Notes:**



